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Inhibitory effects of Aconiti Lateralis Radix Preparata on chronic intermittent cold-induced inflammation in the mouse hypothalamus

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ABSTRACT

Ethnopharmacological relevance: Aconiti Lateralis Radix Preparata (AR) is the most frequently used herb to generate heat and treat symptoms associated with coldness in Asia.
Aims of the study: The hypothalamus is one of the master regulators to maintain constant core body temperature. Chronic exposure to cold stress disturbs homeostatic regulation, gradually resulting in hypothalamic inflammation. This study investigate the effects of AR, on the chronic intermittent cold (CIC)-induced release of pro-inflammatory signaling molecules in the mouse hypothalamus.
Materials and methods: Aconiti Lateralis Radix Preparata extract (ARE) were solubilized in distilled water and diluted with saline before administration. Male ICR mice (7 weeks old, 30–32 g) were divided randomly into 6 groups: (1) control, (2) cold stress, (3) ARE 30, (4) ARE 100, (5) ARE 300, and (6) ARE 1000 mg/kg groups. Groups (2)-(6) were exposed to CIC stress once a day for 14 days. CIC stress was achieved by exposing the mice to 4 °C and 60 ± 10% humidity for 120 min once a day. Rectal temperature was measured after terminating cold stress. Cortisol levels were measured from serum. Hypothalamus tissue was used for western blot analysis, and IL-9, IL-13, PGE1, and PGE2 levels were assessed.
Results: ARE treatment prevented the CIC-induced decrease in rectal temperature and increase in serum cortisol level. ARE-treated CIC-exposed mice demonstrated decrease in nuclear c-Fos levels dose-dependently compared to CIC-exposed mice. Nuclear NF-kB expression showed significant increase in CIC-exposed mice. ARE treatment significantly blunted the increase in nuclear NF-kB expression. CIC-exposed mice had significantly increased levels of both IL-9 and IL-13. Treatment with ARE suppressed the elevated IL-9 and IL-13 levels. Between control and CIC-exposed mice PGE1 levels showed no difference. However ARE (1000 mg/kg)-treated CIC-exposed mice had a significant increase in PGE1 level compared to CIC-exposed mice. PGE2 levels were significantly higher in CIC-exposed mice compared to control mice. ARE treatment significantly attenuated the increase in PGE2 levels.
Conclusions: Our findings suggest CIC stress disturbs the anti-inflammatory effect of cortisol and maintenance of the body temperature. Thus AR contributes to suppress the activated proinflammatory factors, IL-9, IL-13, and PGE-2, and to increase the heat production.

1. Introduction

Homeostasis is a fundamental property of a living organism. It preserves their stability within an acceptable range in order to adapt to a broad range of external environments (Ganong, 2005). Homeostatic regulation is well defined and includes not only the maintenance of the core body temperature but also of the blood glucose levels, blood pressure, and circadian rhythms (Makino et al., 2009). Being exposed to cold temperatures stimulates cold sensing receptors and activates the hypothalamus, thereby resulting in vasoconstriction, an increase in

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metabolic rates and shivering thermogenesis to maintain constant core body temperature (Makino et al., 2009). However, chronic exposure to cold stress disturbs homeostasis, maintained by endocrine and autonomic nervous systems, gradually resulting in an unstable internal environment and increased risk of illnesses (Makino et al., 2009).

Exposure to chronic intermittent cold (CIC) stress increases the reactivity of the hypothalamic-pituitary-adrenal (HPA) axis to subsequent acute stressors as measured by plasma adrenocorticotropic hormone (ACTH) and corticosterone levels (Ma and Morilak, 2005; Pardon et al., 2003). Cortisol may modulate transcription of cortisol-responsive genes, thus decreasing the production of cytokines and other inflammatory mediators (O'Connor et al., 2000). For example cortisol directly decreases transcription of interleukin-6 (IL-6) and interleukin-1β, and indirectly suppresses pro-inflammatory transcription factors such as nuclear factor-κB (NF-κB) and activating protein-1 (AP-1) (Barnes, 1998). However, activation of NF-κB in the nucleus, binds to specific sequences in the promoter regions of target genes and increases the expression of many cytokines, enzymes, and adhesion molecules in inflammatory diseases (Barnes and Karin, 1997). Morilak's group used the CIC stress to study the brain function, and reported that CIC stress sensitizes stress hormone reactivity and deficit in cognitive flexibility (Girotti et al., 2011). A mouse model of CIC stress has also been shown to be useful for studying chronic muscle pain, atherosclerotic plaque instability, tactile allodynia, ovarian insulin resistance, and ovarian follicular development (Akagi et al., 2014; Dorfman et al., 2003, 2009; Nasu et al., 2010; Zheng et al., 2014). Moreover CIC stress was used to examine biological effects of herbs used in Traditional Chinese Medicine (TCM) to attenuate brain oxidative damage, hypothermia and immune suppression (Kim et al., 2013; Makino et al., 2009).

Aconiti Lateralis Radix Preparata (AR), the processed daughter root of *Aconitum carmichaeli* Debx., family Ranunculaceae, has been used widely in Asia as an essential herbal drug for cold sensation by stimulating heat generation (Suzuki et al., 2016). Traditionally AR has been used as a cardiotonic, analgesic, anti-inflammatory, and diuretic agent for thousands of years to treat colds, polyarthralgia, diarrhea, heart failure, beriberi, and edema (Murayama et al., 1991). Nowadays, in clinic, AR is commonly used for diarrhea, syncope, joint pain, rheumatoid arthritis and other inflammations (Chen et al., 2015). Many studies have shown that AR has anti-inflammatory, anti-tumor, and analgesic effects (Bai et al., 2008).

Up to date, little is known about the involvement of AR in the stress induced inflammation response in the hypothalamus. The involvement of a signal pathway that regulates the release of pro-inflammatory factors of hypothalamus by medicinal herbs in a CIC stress model has not been reported. Interestingly, a recent study has reported that oral administration of processed aconite root ameliorated cold stress-induced decrease in core body temperature and natural killer cell activity by up-regulation of UCP1 expression in brown adipose tissue (Makino et al., 2009). The aim of the present study was to investigate the effects of AR on the CIC-induced release of pro-inflammatory signaling molecules in a mouse model.

2. Materials and methods

2.1. Materials

Rabbit anti-c-Fos and cortisol enzyme-linked immunosorbent assay (ELISA) kits, prostaglandin E1 (PGE1) and prostaglandin E2 (PGE2) kits were purchased from Enzo Life Sciences (Farmingdale, NY, USA). Goat anti-heat shock protein 70 (HSP70), rabbit anti-nuclear factor (NF)-kB and mouse anti-β -actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Mouse anti-proliferating cell nuclear antigen (PCNA) was purchased from BD Transduction Laboratories (Franklin Lakes, NJ, USA). Ethanol and phosphate buffered saline (PBS) were purchased from Sigma–Aldrich (St. Louis, MO, USA). A protein assay kit was purchased from Bio-Rad Laboratories (Hercules, CA, USA). Interleukin (IL)-9 and IL-13 ELISA kits were purchased from Ray Biotech (Norcross, GA, USA). The Nuclear/Cytosol Fractionation Kit was purchased from BioVision (Milpitas, CA, USA).

2.2. Preparation of ARE

Aconiti Lateralis Radix Preparata (AR) was purchased from Omni Herb Inc. (Andong-si, Gyeongbuk, Korea). Two hundred milligram of air-dried products were cut into smaller pieces and pulverized to dry powder, respectively. The fibrous powder of the AR were extracted in boiling distilled water (DW) for 2 h, respectively. Mixed-DW extracts were filtered through Whatman No. 1 paper and concentrated to dryness under reduced pressure in a rotary evaporator. The lyophilized ethanol extracts were successively extracted with a yield (w/w) of 21.1% (AR), respectively. The lyophilized powders (Aconiti Lateralis Radix Preparata extract; ARE) were solubilized in DW and diluted with saline before administration. The reproducibility of AR was confirmed by producing a fingerprint using reference compound: aconitine for AR, respectively (Jung et al., 2014). The AR extract (Voucher No. AR2014-01) samples were deposited at the College of Pharmacy, Kyung Hee University for future use.

2.3. Standardization

The AR at 100 mg/ml was applied on quantitative chemical analysis of aconitine, a standard compound of *Aconitum carmichaeli* Debx by high-performance liquid chromatography (HPLC). AR sample was dissolved in 0.01 N hydrochloric acid at 100 mg/ml, centrifuged at 3000 rpm for 5 min, and filtered using 0.45 μm PVDF membrane (Whatman). Aconitine was purchased from Sigma (St. Louis, MO, USA) as HPLC grade. This method was modified as a described method (Jiang et al., 2005). AR was standardized based on aconitine content determined using a Waters 2998 HPLC system (Waters Corp., Milford, MA, USA). The chromatographic separation was performed by using an Atlantis C18 column (250 × 4.6 mm, 5 μm) and column temperature was maintained at 40 °C. The mobile phases (A: CH_3CN, B: 1.2 g NH_2PO_4 in 1000 ml purified water + adjust pH 3.0 with phosphoric acid) were 95% B for 0–5 min, 95–50% B for 5–40 min, and 50–10% B for 40–60 min. Chromatography was carried out in gradient mode using a flow late of 1.0 ml/min. The chromatograms were detected at 232 nm using a photodiode array (PDA) detector (Waters Corp., Milford, MA, USA). Data acquisition was performed with Empower 3 software (Waters Corp., Milford, MA, USA).

2.4. Animals and cold exposure

Male imprinting control region (ICR) mice (7 weeks old, 30–32 g) were obtained from the Orient Co., Ltd. (Seoul, Korea). Mice were randomly assigned into ten groups: (1) control, (2) cold stress, (3) ARE 30, (4) ARE 100, (5) ARE 300, (6) ARE 1000 groups. Groups 2–6 were exposed to cold stress once a day for 14 days, respectively. The animals were housed 10 per cage (40 × 25 × 18) and maintained under a 12-h light/dark cycle at constant temperature (23 ± 1 °C) and humidity (60 ± 10%) with free access to food and water. Animal maintenance and treatment were carried out in accordance with the Animal Care and Use Guidelines issued by Kyung Hee University, Seoul, Korea (approved number; KHP-2014-05-3). Mice were habituated to their new environment and handled for 1 week after arrival, and kept under the same conditions before starting the study. To avoid the influence of diurnal cycling, all experiments began at approximately the same time each day. Cold exposure was achieved by putting the animal's home cage into a Misco control system (Samsung, Seoul, Korea) maintained at 4 °C and 60 ± 10% humidity for 120 min once a day. Changes in body weight were measured using an electronic balance (OHAUS Corporation, Parsippany, NJ, USA). Changes in body temperature were